BIOLOGY OF PLANT-MICROBE ASSOCIATIONS

Panel Manager – Dr. Steven Pueppke, University of Illinois
Program Director – Dr. Ann Lichens-Park

Research Grants in this program support studies aimed at understanding the biology of microorganisms, the interactions between microorganisms and plants, the effects of microbes on plant biology, and the influence of biotic and abiotic environmental factors on plant-microbe interactions. Studies may focus on microorganisms that have detrimental effects on plants, such as plant pathogens, or on microorganisms that have beneficial effects on plants, such as nitrogen-fixing bacteria.

2001-02684 Bacterial Cross-communication: Role in Gene Expression, Survival and Pathogen Inhibition

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Grant 2001-35319-10963; $300, 000; 3 Years

Plant-associated bacteria play major roles in agricultural plant health, whether the bacteria benefit the plant (such as plant growth-promoting rhizobacteria, nitrogen fixing bacteria), cause plant damage or disease (such as pathogenic bacteria), or live saprophytically (aid in plant nutrient cycling). In nature, bacteria rarely (if ever) exist as single-species populations and must interact both cooperatively and competitively with other members of the microbial community. For any plant-microbe interaction to benefit the plant, the bacterium must persist as a component of the indigenous microbial community. It is crucial that we understand the interactions that determine plant-associated bacterial community structure and composition. We will use the well-characterized root-colonizing biological control bacterium *Pseudomonas aureofaciens* as a model to study the influence of the indigenous microbial root community on the expression of genes that encode products critical for survival and inhibition of plant pathogenic fungi. We have found that the community contains different subpopulations of microbes that positively or negatively interact with *P. aureofaciens* and affect its ability to suppress disease. We previously characterized some of the genetic and ecological mechanisms involved in positive interactions. Now we will focus on the mechanisms involved in negative interactions as these could explain the variability in plant-associated bacterial disease control. Negative interactions will be analyzed using both genetic and natural product approaches. Our goals are to understand the effect of negative interactions on bacterial gene expression, to understand how subpopulations can affect the ecology of plant-associated bacterial communities, and ultimately to improve plant health

2001-02737 Biochemical Isolation of Bs2 and AvrBs2 Interacting Proteins

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Postdoctoral Fellowship; Grant 2002-35319-11588; $90,000; 2 Years

Plants are constantly under attack by phytopathogenic organisms; however the outcome of most of these plant-pathogen interactions is resistance whereas the

development of disease is the exception. Essential to the plants defense strategy is the rapid recognition of the invading pathogen and the activation and expression of resistance. The understanding of the initial events leading to disease or the activation of the resistance response is essential for maintaining an adequate food supply. Bacterial spot is a devastating disease of pepper and tomato caused by the bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*). *Xcv* is thought to deliver virulence factors into plant cells by the type III protein secretion system. Once inside the plant cell, the virulence factors interact with the plant cell in order to aid in the infection process. Resistant plants have evolved mechanisms to recognize these foreign proteins. Recognition of these foreign proteins initiates a signal transduction cascade leading to the activation of the resistance response. The goal of this project is to use a biochemical approach to identify factors involved in the early events of virulence and to elucidate the mechanism by which plants detect these virulence factors and activate the signal transduction pathway leading to the resistance response.

2001-02748 Function of Cloned Avirulence Genes in *Phytophthora sojae* Infection of Soybean

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Grant 2001-35319-10962; $230,000; 2 Years

Oomycetes are fungus-like organisms that are actually relatives of brown algae such as kelp. Oomycetes cause disease on almost all crop plant species, causing enormous economic damage - tens of billions of dollars worldwide every year. The overall goal of this research is to characterize genes involved in infection by the organism that causes Phytophthora root rot of soybean, an oomycete called *Phytophthora sojae.* Pathogen genes called avirulence genes are often used by pathogens to aid in attacking the plant, but are also used by the plant's defense machinery as a signal that a pathogen is present. As a result, understanding the precise role of these genes in disease is key to improving strategies for protecting plants against pathogens. We have cloned an avirulence gene from *P. sojae* called *Avr1b*. In this project we will characterize the gene in more detail and the protein it encodes. In particular we will focus on how the protein contributes to the susceptibility of soybean plants by *P. sojae*.

2001-02678 Mechanism of Gene Silencing Suppression by the Cucumber Mosaic Virus 2b Protein

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Grant 2002-35319-11537; $242,000; 3 Years

Post-transcriptional gene silencing is a novel cellular pathway that provides protection against viruses in plants by a specific degradation of RNA. Remarkably, localized RNA silencing leads to production of a signal, which can spread and direct specific RNA silencing at a distance. The cucumber mosaic virus 2b protein (Cmv2b), required for virulence determination and systemic virus spread, was first identified as a suppressor of transgene RNA silencing in 1998. Based on our recent and previous data, we propose that Cmv2b suppresses RNA silencing by preventing transport of the silencing signal from the primarily silenced cells to non-silenced cells and/or inhibiting

initiation of the RNA silencing pathway in the non-silenced cells after entry of the signal. This proposed role of Cmv2b in interfering with the functionality of the signal will be examined using four complementary experimental systems based on Agrobacterium infiltration and grafting techniques. In addition, we will determine (i) if Cmv2b suppression leads to a reduced accumulation of the 25-nucleotide RNA's and a decreased transgene DNA methylation and (ii) if Cmv2b is able to suppress virus-induced RNA silencing. Moreover, a mutational analysis will be carried out to determine domains important for, and to explore the possibility of uncoupling, the various activities of Cmv2b in silencing suppression. Cmv2b represents a unique type of viral silencing suppressors as it reverses silencing only in the newly emerged tissues. Thus, the work proposed here will likely reveal a distinct mechanism used by plant viruses to suppress the RNA silencing antiviral pathway.

2001-02733 Quantitative Evaluation of the Role of Water Stress in Bacterial Colonization of Leaves

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Grant 2001-35319-10929; $275,000; 3 Years

Leaf-associated bacterial populations can influence plant health in several ways, including altering growth through plant hormone production and inciting as well as protecting plants from disease and frost injury. The sizes of these populations are often related to these effects; thus, an understanding of the factors influencing bacterial leaf colonization should contribute to strategies for managing these populations to benefit plant health.

The goal of this study is to identify the quantitative contribution of bacterial-water relations to bacterial population dynamics on leaves. Bacterial exposure to water deprivation will be quantified using newly developed biological sensors. The first objective of this study is to identify the relationships among the levels of bacterial exposure to water deprivation on leaves, the patterns of bacterial growth and death over time, and the macroenvironmental conditions during bacterial leaf colonization under field conditions. The second objective is to identify the relative impact of plant species and specific environmental conditions on bacterial exposure to water deprivation on leaves, as well as the effect of various water deprivation levels on bacterial growth and survival in culture.

2001-02618 Lipid Signaling and NPR1-independent Pathway in Plant Defense

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Grant 2002-35319-11655; $131,000; 2 Years

Diseases severely limit plant productivity and quality. Fungicides and pesticides target a broad-spectrum of pathogens and pests. However, this kind of chemical control is expensive and harmful to the environment and public health. Plants have preformed and inducible defense mechanisms. Strategies of protection that augment a plants innate ability to resist a wide spectrum of pathogens provide a feasible alternative to chemical control of plant disease. Hence, identifying the defense mechanisms that are activated leading to resistance are important for developing new cost effective and environmentally

sound strategies of plant protection. The *Arabidopsis thaliana* NPR1 protein is a key regulator of plant defense. However, NPR1-independent mechanisms are also important to plant defense. Activation of both these pathways is essential for complete resistance. Very little is known about the components and functioning of these pathways. The *SSI2* gene is a component of the NPR1-independent defense pathway. Mutations in the Arabidopsis *SSI2* gene disrupt lipid biosynthesis and alter plant defense responses. We propose to use the power of genetics to further evaluate the role of lipid signaling in plant defense. Interaction of the *ssi2* mutant with other genes regulating defense responses will be studied. Other components of lipid signaling pathway in plant defense that have been identified using genetic screens for suppressors of the ssi2 mutant will be cloned and characterized. Identifying the components and understanding the mechanism of signaling through this uncharacterized resistance pathway will assist in devising alternative strategies of plant protection.

2001-02621 General Resistance Mediated by Arabidopsis NHO Genes and Pseudomonas Virulence

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A given plant species is resistant to the majority of plant pathogens, and a given phytopathogen only causes diseases on a limited number of plant species. This type of resistance is referred to as non-host resistance. In other words, non-host resistance is a form of durable resistance that widely exists in plants. The durable nature or non-host resistance is extremely important to agriculture, because most disease resistance genes deployed in breeding programs only provide short-lived resistance that are often broken down by new races of pathogens. However, non-host resistance is poorly understood and difficult to use. The PI 's work using Arabidopsis and Pseudomonas bacteria as a model system has identified a number of non-host resistance genes, called NHO genes, in Arabidopsis that can be genetically manipulated. Some of these genes control resistance to different bacterial pathogens and fungi. In this project, the PI will clone two NHO genes, NHOI and NH02, from Arabidopsis plants. The PI will also attempt gene transfer between different plant species to test if the non-host resistance genes isolated from one plant species can be utilized to protect crop plants from a wide range of diseases. This represents one of the first attempts to isolate non-host resistance genes and understand how they work in plants. It is expected that the work proposed here will provide useful tools and knowledge base to engineer durable resistance in crop plants. The work will also encourage other scientists to take a parallel approach to isolate non-host resistance genes from other plant species.

2001-00757 Enhancing Genetic Analysis and Sequencing Facility

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Efficient elucidation of fundamental biological questions relevant to agriculture requires efficient deciphering of information inherent in cells of plants, animals and microorganisms. That information is in the form of deoxyribonuclcic acid (DNA, the

chemical code of the genes that direct synthesis of ribonucleic acids and proteins, which, in turn, carry out biological functions including an establishment of cell and organism architecture, as well as critical biochemical reactions. The purpose of the project is to institute a facility for high-throughput DNA sequencing, then to use that facility for detailed genetic analysis of agriculturally relevant organisms. Among the projects this facility will serve are studies of symbiotic fungi and viruses that play a significant role in biological control of agricultural pests, molecular control of plant developmental processes, DNA analysis of animal parasites to identify potential diagnostic and therapeutic targets, and elucidation of changes in genetic information of plant viruses that lead to entities that help suppress or prevent symptoms of viral diseases. The requested instrumentation will significantly increase productivity and performance of these research programs and will greatly facilitate development of other research programs in the College of Agriculture at the University of Kentucky.

2001-02617 Microarray Analysis of the Susceptible Interaction between *Arabidopsis* and *Pseudomonas syringae* pv. *tomato* DC3000.

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Postdoctoral Fellowship; Grant 2002-35319-11558; $90,000; 2 Years

The long-term goal of this research is to increase our understanding of the molecular mechanisms underlying plant host susceptibility to bacterial infection. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) causes disease on both *Arabidopsis thaliana* and tomato plants. The *Pst* bacteria produce virulence factors (toxins and secreted proteins) which contribute to disease development on susceptible host plants. I will examine the *Arabidopsis* host gene expression following *Pst* inoculation using DNA microarrays. The following specific objectives will be pursued: 1) identify *Arabidopsis* genes with altered expression in response to individual or multiple *Pst* virulence factors; 2) characterize *Pst*-regulated *Arabidopsis* genes using defense compromised *Arabidopsis* mutants; and 3) initiate a study of the function of selected *Pst*-regulated *Arabidopsis* genes by gene knockout and/or overexpression in transgenic plants. Knowledge gained from the proposed research will contribute to our understanding of the molecular aspects of susceptible plant-pathogen interactions. This work may identify important candidate genes that can be utilized to decrease the susceptibility of the host plant to pathogen attack and potentially be used for the genetic improvement of agriculturally important crop plants.

2001-02700. The Mechanism of Forcible Discharge of Ascospore in *Gibberella zeae*.

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Grant 2001-35319-10898; $133,916; 2 Years

The fungi in the Phylum Ascomycota are characterized by the production of their sexual spores, ascospores, in a sac-like structure called an ascus (pl. asci). Many of the most devastating fungal plant pathogens are in the Phylum Ascomycota and, for some, airborne ascospores are the primary inoculum for the diseases they cause. The ascus evolved as a tubular gun used to shoot ascospores into the air. Although genera of the

Ascomycota have long been used as models for genetic studies, the genetics and physiology of the forcible discharge mechanism has not been elucidated. Our long-term goal is to understand the mechanism whereby ascospores are forcibly discharged from asci.

We propose to begin to explore the physiological and genetic basis for Ascospore discharge in *Gibberella zeae*, the causal agent of head blight of wheat and barley. It is likely that this mechanism is conserved over the Ascomycota and an understanding will be applicable to many Ascospore-producing pathogens. Our approach uses physiological, genetic and genomic resources. We have identified components in the ascus sap that are likely involved in the discharge process. The genes involved in the generation of these components will be cloned, and their role in discharge assessed. A mutant that has lost its ability to discharge ascospores will provide us with another approach towards characterizing the factors involved in discharge. An understanding of the mechanism of discharge will lead to development of control methods that prevent discharge, and therefore reduce or eliminate the primary inoculum of these important pathogens.

2001-02707 13th International Congress on Nitrogen Fixation

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Grant 2001-35303-11007; $10,000; 1 Year

Nitrogen (N_2) fixation is a process by which atmospheric N_2 gas is converted into a fertilizer form capable of being used by all organisms. The process occurs most notably in legume plants when growing in symbiosis with a group of soil bacteria collectively called rhizobia. Nitrogen fixation is important to agriculture for three crucial reasons: 1) legume plants obtain most of their N needs from this process; 2) it is a readily renewable, sustainable source of N fertilizer; and 3) it provides about 35% of humankind's N needs. Funds are requested in this proposal to support the registration costs of U.S. scientists attending the International Congress on Nitrogen Fixation held in Hamilton, Ontario, Canada. The meeting addresses current advances in N_2 fixation and keeps U.S. scientists abreast of rapidly changing information. Basic and applied advances in the field hold potential for reducing energy requirements in U.S. agriculture.

2001-02619 Geminivirus Rep proteins: Host Interactions and Disease Resistance

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Grant 2001-35319-10856; $275,000; 3 Years

Geminiviruses are a large family of DNA viruses that infect a broad range of plant species. In recent years, they have emerged as leading plant pathogens that cause severe crop losses in the United States and worldwide. Unfortunately, very few control methods or geminivirus resistant cultivars are available to combat the disease problems. As a consequence, development of a rationale strategy to combat geminivirus disease is imperative. We will test a dual strategy toward geminivirus resistance directed at the viral replication proteins, Rep and C3. Under Aim 1, we will construct transgenic plants that express mutant Rep proteins that interfere with viral replication but do not bind to the host factor, retinoblastoma - a regulator of plant cell division and development. These plants will be tested for stable Rep expression and disease resistance. Under Aim 2, we

will develop a co-expression system for Rep and C3 mutants and assess the ability of plants expressing both mutant viral proteins to resist infection by a range of Geminiviruses. These experiments are based on preliminary studies suggesting that expression of either a Rep or a C3 mutant in transgenic plants leads to disease attenuation, and that Rep and C3 mutants act synergistically to further reduce disease. Together, these studies will serve as basis for the rapid generation of disease resistant crops that are targeted by single or multiple Geminiviruses.

2001-02690 Molecular Evolution of the Irish Potatoe Famine Pathogen *Phytophthora infestans*

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Grant 2001-35319-10855; $196,000; 3 Years.

Late blight caused by the *Phytophthora infestans* causes a devastating disease that in 1845 led to the Irish potato famine and the mass migration and death of over 3 million people in Ireland. A study by scientists at North Carolina State University, published in the June 7 issue of the journal *Nature*, finds that the strain of the pathogen blamed for the famine is not the guilty culprit after all. DNA fingerprinting analysis of 150-year-old leaves, preserved from the Irish potato famine, found no trace of the widely suspected 1-b haplotype (strain) of the late-blight pathogen *Phytophthora infestans*. We will use mtDNA and nuclear DNA gene sequences to unravel the genealogical history of the potato late blight pathogen and ask the following questions: 1) What ancestral strain was responsible for late blight epidemics that caused disease in 1845 in Ireland and Europe? Did the initial migrations of *P. infestans* into Europe, Ireland, and England in the 1840's contain one or multiple mtDNA types of *P. infestans* ? 2) Does mtDNA and nuclear DNA gene sequence evidence justify the specific hypothesis that a common ancestor of *P. infestans* originated in South American populations? This hypothesis will be tested by examining gene sequence evolution in the pathogen by construction of phylogenetic trees for several genes of interest in the mitochondrial and nuclear genome in a range of isolates from a modern collection from different geographical areas in the US, Ireland, UK, Europe, Mexico, Central and South America, and in samples from historic herbarium specimens.

2001-02751 Coordinated deployment of effector proteins by *Pseudomonas syringae pv. tomato* DC3006

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Postdoctoral Fellowship; Grant 2002-35319-11557; $90,000; 2 Years

Pseudomonas syringae is a bacterium that causes brown lesions on the leaves of various plants including tomato, bean, and tobacco. The ability of *Pseudomonas syringae* to infect plants appears to depend upon proteins that are injected directly into plant cells by a specialized bacterial secretion apparatus known as a type III secretion system. The secreted bacterial proteins may allow *P. syringae* to cause disease by promoting bacterial growth in the plant or by suppressing plant defense responses. This project aims to test the idea that *P. syringae* must coordinate the relative delivery of these virulence proteins to effectively parasitize its hosts. The expression, secretion, and delivery of multiple *P.*

syringae type III secreted proteins will be compared at various times during *P. syringae* growth in culture or in plants. This research may provide insights into the molecular mechanisms underlying bacterial parasitism of plants.

2001-02691 AHL Signaling in *Sinorhizobium meliloti* and the Effects of AHL Signal Mimic Compounds from its Host *Medicago truncatula*

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Grant 2002-35319-11559; $210,000; 2 Years

The research has two general objectives: to characterize the *N-acyl* homoserine lactone (AHL)-mediated quorum-sensing system of the N-fixing bacterium *Sinorhizobium meliloti* and to determine how the AHL signal-mimic compounds produced by its legume host, *Medicago truncatula*, affect gene expression in the bacterium. The AHL signaling system of *S. meliloti* will be characterized by chemically identifying the AHL signal compounds, and by identifying the enzymes that synthesize these AHLs and the receptors that respond to them by mutating genes identified through the genomic database as likely to have these functions. Proteomic and random mutagenic analysis will be used to identify AHL-regulated genes. Several AHL signal-mimic compounds from *M. truncatula* will be purified and tested to determine their effects on gene expression in *S. meliloti*, both through proteomic comparisons and through use of luminescence reporter insertions in genes regulated by AHLs. Careful characterization of the AHL signaling system in S. *meliloti* will prove to be of fundamental value to the plant-microbe interaction field as a whole and be an essential part of learning how the AHL signal-mimics from *M. truncatula* affect this bacterium and its symbiotic interactions. These two model organisms provide a promising system for learning about AHL signal-mimics from plants and advancing these discoveries rapidly at the genetic, genomic and biochemical levels. In the long term, these studies may contribute significantly to the manipulation of signal-mimics in crop plants to enhance plant health by better symbioses and increased resistance to pathogens.

2001-02631 A Signaling Cascade that Regulates *hrp* Pathogenicity Genes in *Pantoea* spp.

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Grant 2002-35319-11562; $210,000; 3 Years

Plant pathogenic bacteria employ specialized "Hrp" secretion systems to deliver an arsenal of pathogenicity proteins directly into host cells. *Pantoea stewartii* causes a serious disease of maize and sweet corn, known as Stewart's Bacterial Wilt and Leaf Blight. We have characterized a *hrp* gene cluster in *P. stewartii* and demonstrated that it is regulated in response to environmental, nutritional and plant signals by the following signal transduction cascade: 1) Environmental and/or physiological signals are sensed by the HrpX protein. 2) HrpX phosphorylates HrpY changing it into a transcriptional activator that turns on the *hrpS* gene. 3) HrpS then turns on *hrpL*. 4) Finally, the HrpL protein enables RNA polymerase to recognize the promoters of other *hrp* genes. This system has a number of unusual features: HrpX may sense the energy state of the bacterium; a novel methylated receptor protein may modulate HrpX activity in response

to host cell contact; and *hrpS* expression appears to be regulated by population density. The overall goals of this project are to genetically and biochemically characterize the regulation of the *hrpS* promoter, which is a key point in integrating many different signals, and to determine how the HrpX/Y two component system functions in pathogenesis. *hrp* gene regulation may provide a target for plant-produced compounds that interfere with recognition of the host by the pathogen. Using information from this project, plant genetic engineers may be able to create plants that are resistant to a broad range of bacterial diseases.

2001-02723 Analysis Soilborne wheat mosaic virus cell-to-cell and vascular transport

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Grant 2001-35319-10897; $181,000, 3 Years

Soilborne wheat mosaic virus (SBWMV) causes disease in winter wheat in the central and southern plains regions of the U.S. and can cause as much as 80% yield loss. In spite of its agronomic importance there has been very little research exploring the requirement for SBWMV infectivity. This research is important for developing methods to control virus infection. SBWMV is transmitted to wheat roots by a fungal vector. Recently we determined in my laboratory that the virus is likely to move long distance through the xylem to reach aerial portions of the plants. This is surprising since most viruses utilize the phloem for long distance transport. Experiments outlined in this proposal are designed to determine the role of viral proteins in facilitating viral intercellular and vascular transport. In addition experiments will be conducted to explore the natural path for virus movement into the vasculature following inoculations of wheat roots or leaves. We will use transgenic wheat plants expressing individual viral genes for complementation studies. We will also develop a system to culture the fungal vector in wheat roots.

2001-02623 Host Responses to Potyviruses

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Grant 2002-35319-11560; $300,084; 3 Years

This project focuses on the host factors and functions that confer susceptibility to viruses. Identification and understanding of these factors and functions is necessary for long-term development of sustainable crops and agricultural products. A genetic approach is employed to identify mutants of *Arabidopsis thaliana*, a model plant, that have lost susceptibility to turnip mosaic potyvirus, a model plant virus. These mutants, termed loss-of-susceptibility to potyviruses (*lsp*), are unable to support virus replication or virus systemic movement. These mutants enable isolation of genes that play important roles in the virus infection process or in defense against viruses. In particular, the effects of these mutations on the RNA silencing defense system will be determined. This project has two Specific Objectives: 1) Characterize a series of Arabidopsis *lsp* mutants, which

have defects in two distinct genes (*lsp1* and *lsp2*), and 2) isolate both *LSP1* and *LSP2* genes using map-based cloning procedures.

2001-02692 Multilevel Organization of the Closterovirus Machinery for Cell-to-Cell Movement

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Grant 2001-35319-10857; $300,000; 3 Years

This project is concerned with the molecular mechanisms of the closterovirus transport inside the infected plants. Closteroviruses are the large family of pathogens affecting production of a number of crops such as vegetables, citrus, and grapes. Along with the geminiviruses and tospoviruses, closteroviruses are responsible for the explosion of newly discovered viral diseases. In agriculture, closteroviruses are transmitted by insects that introduce virus into the plant vascular system. Virus reproduction and transport between cells results in colonization of the entire vascular system, severe disease, and crop loss. Despite the growing economical importance of closteroviruses, studies of their molecular biology were long impeded by difficulties of working with the unusually long and unstable thread-like virus particles. Recent success in application of recombinant DNA technology permitted us to address the mechanisms of closterovirus replication, spread, and pathogenicity, and to identify viral proteins functioning in these processes. We have developed a conceptual model that suggests integration between processes of closterovirus replication, particle assembly, suppression of host defenses, and movement from cell to cell. The major goal of this project is to test this model and to reveal functional and spatial organization of the closterovirus machinery for cell-to-cell movement. This will be done using advanced methods of genomics, proteomics, and cell biology. The information obtained will contribute to solving a fundamental problem of virus-host interactions, help to improve closteroviral gene expression vectors for application in biotechnology, and assist in designing novel strategies for closterovirus control.

2001-02760 Silencing *Agrobacterium tumefaciens* oncogenes

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Grant 2002-35319-11555; $270,000; 3 Years

Crown gall tumors result from overproduction of growth hormones (auxin and cytokinin) in plant cells transformed by *Agrobacterium tumefaciens*. These abnormally high phytohormone levels result from expression of three genes transferred stable into the plant genome from the *A. tumefaciens* tumor-inducing plasmid. We introduced defective copies of these genes into plants and discovered that they can render the plants resistant to gall formation. These transgenic plants prevent crown gall disease by silencing the wild-type oncogenes transferred from *A. tumefaciens*. We discovered that the sequences of target genes influence the efficiency of gene silencing. Our present work will identify the sequences that trigger gene silencing most effectively. Adequate means do not exist to control crown gall on grapes, fruit and nut trees, and other nursery crops. This work

will lead to production of crop plants resistant to crown gall and allow us to understand why only some target genes are silenced readily.

2001-0263 5 Genetic Dissection of Victorin-Induced Responses in Arabidopsis
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Grant 2001-35319-10896; $299,000; 3 Years

Despite obvious differences in the outcome of plant resistance and susceptibility responses, there are indications that these seemingly distinct responses share characteristics and that a significant determinant of the disease outcome is the pathogen's reaction to the host's response. Similarities of disease resistance and susceptibility responses, and the role of the pathogen in dictating these outcomes, are nowhere more evident than in the disease, Victoriae blight of oats which is caused by the fungus *Cochliobolus victoriae*. *Cochliobolus victoriae* produces a selective toxin, victorin, which is causal to disease development in sensitive oats, and the gene conferring victorin sensitivity (*Vb*) is genetically inseparable from a rust resistance gene, Pc-2. In addition to conditioning susceptibility, victorin also elicits a host response that shares characteristics with defense responses including programmed cell death (PCD), and is therefore similar to race-specific elicitors of a plant defense response called the HR. Recently, we discovered victorin sensitivity in *Arabidopsis thaliana*, which, as in oats, is conditioned by a single dominant gene we have named *AVb*. *AVb* provides a unique opportunity to investigate relationships among PCD, defense responses, and susceptibility in a genetically tractable, model plant, *Arabidopsis*. We propose to genetically dissect the victorin-induced cell death pathway with defense/cell death mutants of *Arabidopsis*, and by generating mutations that suppress *AVb*. We will also define biochemical parameters that are shared between victorin-induced and defense responses and investigate the disease reaction of *Arabidopsis* to *Cochliobolus victoriae*. This effort should contribute to our understanding of plant disease and consequently, our ability to manipulate plants to enhance production.

2001-02602 Genetics of Plant Resistance to Pathogenic Bacteria
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Grant 2002-35319-11561; $167,000; 3 Years

The overall goal of this research project is to improve our basic understanding of how plants resist diseases caused by bacteria. This will be accomplished by studying the function of a newly identified plant gene that appears to regulate plant disease resistance responses. This gene is called WRKY. The model plant *Arabidopsis thaliana* (thale cress) interacting with *Pseudomonas syringae* (*P. syringae*) bacteria will be used for this study. *P. syringae* is the causal agent of bacterial speck disease of tomatoes. It also causes bacterial speck disease in thale cress. Thale cress is used as the host for this study because it is relatively easy to identify genes in this organism and the complete structure of the chromosomes is known. The WRKY gene makes the plants more resistant to disease when it is over-activated. We hypothesize that the WRKY gene is controlling

disease resistance responses in the plant. We will use molecular and genetic tools to try to understand the mechanism of the increased disease resistance triggered by over-activation of the WRKY gene. We will also investigate possible practical applications of this knowledge.

2001-02682 Regulation of *Sinorhizobium meliloti* Genes Expressed in Root Nodules
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Grant 2001-35319-10902; $250,000; 3 Years

During the symbiotic interaction between rhizobial bacteria and leguminous plants, such as alfalfa, soybean, and pea, the bacteria induce formation of an organ on the plant roots, called a nodule. The bacterial cells take up residence in the nodule and differentiate into a distinct cell-type called a bacteroid, which is capable of fixing atmospheric nitrogen into ammonia. The ammonia, which is a usable form of nitrogen unlike nitrogen gas, is provided to the plant. The *Rhizobium*-legume symbiosis is important for agriculture because legume crops can be grown without the use of nitrogen fertilizer and usable nitrogen is returned to the soil. The transformation of free-living bacteria into nitrogen-fixing bacteroids is a crucial step for successful symbiosis, but little is known about the developmental pathway at a molecular level. The long-term objective of this work is to understand the molecular and genetic basis of bacteroid formation. The goal during this funding period is to identify regulatory circuits that control bacterial gene expression within the nodule. The work makes use of previously identified genes that are known to be active when bacteroids are formed. The first specific aim is to identify factors that regulate four of these genes. The second specific aim is to identify the DNA sequences upstream of the genes that are used for regulation. Understanding which bacterial genes are needed for bacteroid differentiation and how they are regulated will provide information that is necessary for improving the *Rhizobium*-legume symbiosis or extending host range to other crop plants.

2001-02611 10th Congress on Molecular Plant-Microbe Interactions / 4" Workshop on *Medicago truncatula*
Leong, S.A.
USDA-ARS Plant Disease Resistance Research Unit, Department of Plant Pathology: University of Wisconsin: 1630 Linden Dr.; Madison, WI 53706
Grant 2001-35303-11027; $20,000; 1 Year

Financial support is requested for the 10th International Congress of Molecular Plant-Microbe Interactions as well as the associated 4th Workshop on *Medicago truncatula*. The main Congress will be held from July 10-14, 2001 in the Memorial Union of the University of Wisconsin, Madison USA. The Congress is held under the auspices of the International Society for Molecular Plant-Microbe Interactions (IS-MPMI). The faculty of several departments in the College of Agriculture and Life Sciences of the University of Wisconsin is serving as the local organizing committee. A program has been developed in consultation with the IS-MPMI Board of Directors and several distinguished international scientists. The satellite meeting on *Medicago truncatula* will be held from July 7-9, 2001 immediately before the main Congress. Douglas Cook of the University of California-Davis is organizing this latter event.

Support is sought to provide financial assistance to some of the invited speakers as well as student and conference participants, especially those from developing nations. The main Congress is the premiere venue for communication of new research related to the molecular biology of plant-microbe interactions. Likewise the *M. truncatula* meeting is a key meeting for sharing research findings related to this model legume. Both meetings deal with the basic understanding of how microbes interact with plants in beneficial and deleterious manners. This fundamental science underpins new strategies to protect plants from deleterious organisms and to enhance beneficial interactions that can improve plant productivity. Thus, findings from this field are crucial to the productivity of U. S. agriculture.

2001-02721 Toxin Biosynthesis Genes in Ergopeptine Producing Fungi

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Several fungi in the genus *Neotyphodium* that grow as endophytes (entirely internal to the plant and without inducing visible symptoms) in agronomically important pasture grasses produce toxic ergot alkaloids, primarily ergovaline. Ingestion of ergovaline and other ergot alkaloids results in livestock toxicosis and is a very significant problem in U.S. agriculture. We are studying genes involved in ergot alkaloid biosynthesis as a means to manipulate the accumulation of ergot alkaloids in grass-endophyte associations to determine the alkaloids' roles and, potentially, to ameliorate toxicosis to livestock. We have cloned genes for the first (*dmaW*) and penultimate (*lpsA*) steps of ergovaline production. Using gene knockout technology, we have developed strains of the perennial ryegrass endophyte *Neotyphodium* sp. Lp1, in which either of these two genes has been inactivated. Perennial ryegrass-endophyte associations containing the *lpsA* knockout fungus do not accumulate ergovaline. (The *dmaW* knockouts will be ready for analysis soon.) We propose a series of experiments that will investigate the effects of the ergovaline-eliminating knockout mutations on *Neotyphodium* sp. Lp1-perennial ryegrass associations in terms of animal toxicity, nematode and insect resistance, and agronomic properties. We will concurrently proceed with similar modifications in *Neotyphodium coenophialum*, the economically important endophyte of tall fescue. This project will provide a direct test of ergovaline in a mammalian toxicosis and will indicate the contribution, if any, of ergovaline to several endophyte-associated bioprotective traits. Modified and tested *Neotyphodium* strains may be developed as less-toxic but still bioprotective grass endophytes.